Modulation of *Trypanosoma cruzi*-specific T-cell responses after chemotherapy for chronic Chagas disease

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The aim of this review is to describe the contributions of the knowledge of T-cell responses to the understanding of the physiopathology and the responsiveness to etiological treatment during the chronic phase of Chagas disease. T-helper (Th)1 and interleukin (IL)-10 Trypanosoma cruzi*-specific T-cells have been linked to the asymptomatic phase or to severe clinical forms of the disease, respectively or* vice versa*, depending on the* T. cruzi *antigen source, the patient's location and the performed immunological assays. Parasite-specific T-cell responses are modulated after benznidazole (BZ) treatment in chronically* T. cruzi*-infected subjects in association with a significant decrease in* T. cruzi*-specific antibodies. Accumulating evidence has indicated that treatment efficacy during experimental infection with* T. cruzi *results from the combined action of BZ and the activation of appropriate immune responses in the host. However, strong support of this interaction in* T. cruzi*-infected humans remains lacking. Overall, the quality of T-cell responses might be a key factor in not only disease evolution, but also chemotherapy responsiveness. Immunological parameters are potential indicators of treatment response regardless of achievement of cure. Providing tools to monitor and provide early predictions of treatment success will allow the development of new therapeutic options.*

Key words: T-cells - benznidazole - Chagas disease

Chagas disease, which is caused by the intracellular protozoan parasite *Trypanosoma cruzi*, affects approximately six-seven million people from southern California to South America and Western Europe (Bern et al. 2007, WHO 2015). In 20-30% of infected individuals, this disease results in heart disease or megaoesophagus/megacolon, making Chagas disease the most common cause of infectious myocarditis worldwide (Feldman & McNamara 2000). Early investigations suggested that Chagas disease had an autoimmune aetiology. However, an emerging consensus has indicated that the persistence of parasites might lead to immune exhaustion and altered host immunoregulation, which might be responsible for cumulative tissue damage in chronic Chagas disease (Tarleton 2003, Laucella et al. 2004, Albareda et al. 2006, Gutierrez et al. 2009).

Chemotherapy with nifurtimox or benznidazole (BZ) is recommended during both acute and early chronic phases of *T. cruzi* infection (de Andrade et al. 1996, Sosa-Estani et al. 1998). Several studies have also demonstrated the benefits of chemotherapy with BZ in adults with chronic *T. cruzi* infection (Viotti et al. 1994, Fabbro et al. 2007). Based on this evidence, experts advocate that treatment in adults without advanced heart disease should generally be offered (Bern et al. 2007).

In spite of these latter findings, treatment during the chronic phase of *T. cruzi* infection remains limited primarily because of the lack of early metrics of treatment efficacy and the potential adverse effects of these therapeutics (Viotti et al. 2009).

Due to the low levels of parasitaemia in subjects with chronic *T. cruzi* infections, direct detection of *T. cruzi* or its products or constituents (e.g., DNA, proteins) (Cerisola et al. 1971, Zulantay et al. 2004) is inadequate for determining the effectiveness of treatment, but is useful primarily for indicating treatment failure (Duffy et al. 2013). The primary criterion of a positive response to treatment has been the complete loss of reactivity in serially performed conventional serological tests (i.e., ELISA, haemagglutination and immunofluorescence), as well as the lack of progression to more severe clinical symptoms of Chagas disease. However, the decrease in serological titres using current standard tests is extremely slow, requiring five-10 years to achieve conversion to negative serology in even a fraction of treated adult subjects (Viotti et al. 2006, Bertocchi et al. 2013). Disease progression also occurs over decades and does not occur in all infected individuals, irrespective of treatment (Viotti et al. 2005, Fabbro et al. 2007).

The low percentages of complete seronegative conversion after specific chemotherapy in subjects with long-term chronic *T. cruzi* infections have led to the most likely inaccurate idea that treatment during the chronic phase is useless. Here, we review the contributions of the knowledge of T-cell responses to the understanding of how treatment works with the aim of providing new tools to monitor and predict treatment success during the chronic phase of *T. cruzi* infection.

Function and phenotype of T-cells responsive to *T. cruzi* antigens

The original goals of the studies from our group were to better understand T-cell responses to *T. cruzi* in chronically infected subjects and to correlate these responses with cardiac disease severity. The frequency, phenotype

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and function of T-cells responsive to *T. cruzi* antigens were measured in adults (30-70 years of age) and children (5-16 years of age) with chronic Chagas disease living in areas nonendemic for *T. cruzi* infection in Argentina. The primary findings included an overall low level of detectable *T. cruzi*-specific T-cells and the predominance of single cytokine [interferon (IFN)-γ only]-producing T-cells in the circulation of adult subjects with long-term *T. cruzi* infections (Alvarez et al. 2008) (Table I). Conversely, children presumed to have shorter-term infections showed parasite-specific T-cell responses that were more robust and more highly functional (Albareda et al. 2013) (Table I). The simultaneous secretion of IFN-γ and interleukin (IL)-2 was prevalent among *T. cruzi*-infected children (Albareda et al. 2013), while monofunctional responses were higher in *T. cruzi*-infected adults compared with infected children (Albareda et al. 2006, Alvarez et al. 2008) (Table I). Polyfunctional T-cell responses are important because these cells are optimised for effector function, including higher IFN-γ secretion on a per-cell basis, more efficient killing by IFN-γ and tumour necrosis factor (TNF)-α compared with either cytokine alone and IL-2-mediated expansion of T-cells in an autocrine or paracrine manner, which could enhance T-cell memory function (Seder et al. 2008, Virgin et al. 2009).

Other authors have also reported that children in the early phase of chronic *T. cruzi* infection exhibit IFN-γ and TNF-α-secreting $CD4^+$ T-cells in response to *T. cruzi* antigens (Samudio et al. 1998, Sathler-Avelar et al. 2006); however, these responses were associated with IL-10 production by $CD4^+$ T-cells (Sathler-Avelar et al. 2006). Therefore, the maintenance of functional T-cells is proposed to control parasite replication without the induction of tissue damage and these responses appear to be impaired with the length of the infection. IL-10 might counteract T-helper (Th)1 responses, which, if sustained overtime, might be deleterious for the host (Dutra & Gollobb 2008).

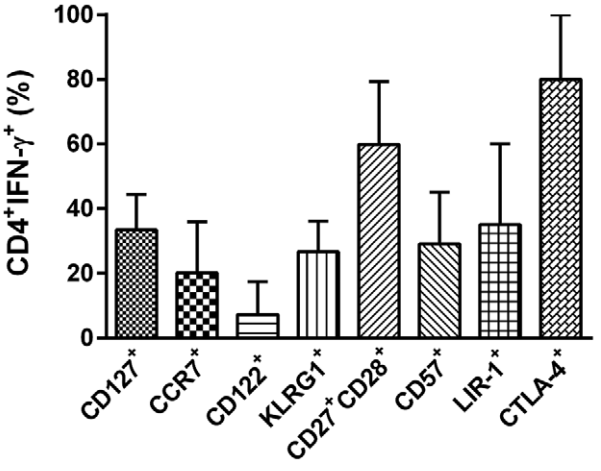
Because phenotypic defects in T-cells might arise along with functional ability loss, the phenotypic profile of *T. cruzi*-specific T-cells has also been evaluated in chronic Chagas disease patients. The pool of parasite-specific IFN-γ-producing $CD4^+$ T-cells is primarily composed of CD122 (the common beta chain of IL-2 and IL-15 receptors) low and chemokine receptor 7 low T-cells, while some of these cells express CD127 (the homeostatic IL-7 receptor), which is consistent with effector memory T-cells (TEMs) (Figure). TEMs that are characteristic of persistent infections have low proliferative capacity and primarily migrate to peripheral tissues; however, in contrast to effector T-cells, TEMs may persist in the host. Furthermore, a fraction of *T. cruzi*-specific T-cells exhibit low expression levels of killer cell lectin-like receptor (KLRG)1, CD57 and leukocyte immunoglobulin-like receptor (LIR)-1 (Argüello et al. 2012), while T-cells expressing the costimulation molecules CD27 and CD28 are enriched (Figure) (Albareda et al. 2006, 2009). Considering that KLRG1 and CD57 expression increases with the rounds of antigen stimulation and that CD27 and CD28 expression is down-regulated (Appay et al. 2008) while LIR-1 expression is up-regulated during T-cell differentiation (Antrobus et al. 2005), these findings indicate that naïve $CD4^+$ T-cells may also be recruited for T-cell responses in chronically infected subjects. Long-term infection with *T. cruzi* also results in the up-regulation of cytotoxic T lymphocyte antigen-4 (Figure) (Argüello et al. 2012) likely as part of a homeostatic process to control tissue damage, although this up-regulation might damper T-cell responses. The above-described findings are compatible with immune exhaustion, which has described for other chronic parasitic and viral infections (Gigley et al. 2012, Rodrigues et al. 2014).

TABLE I

Trypanosoma cruzi-specific T-cell responses in children and adults with Chagas disease living in nonendemic areas of Argentina measured by interferon (IFN)-γ and interleukin (IL)-2 ELISPOT

Positive ELISPOT responses to *T. cruzi* lysate	Positive responders/total *T. cruzi*-infected subjects evaluated n/n (%)	
	Children	Adults
IFN-γ + IL-2	10/17 (58.8)[a,b]	4/22 (18)
IFN-γ-only	3/17 (17.6)[c]	11/22 (50)
IL-2-only	0/17 (0)	0/22 (0)

a: Fisher exact test p = 0.0181 vs. percentage of IFN-γ-only responders in *T. cruzi*-infected children; *b*: p < 0.02 vs. percentage of IFN-γ + IL-2 in adults; *c*: p < 0.05 vs. percentage of IFN-γ-only in adults.



Phenotypic characterization of *Trypanosma cruzi*-specif $CD4^+$ T-cells in chronically infected subjects. Peripheral blood mononuclear cells from eight patients were stimulated with 15 ug/mL of *T. cruzi* lysate from the Brazil strain for 16-20 h. $CD4^+$interferon (IFN)-γ^+ T-cells were selected and analysed for the expression of CD127, chemokine receptor (CCR)7, CD122, killer cell lectin-like receptor (KLRG)1, CD27, CD28, CD57, immunoglobulin-like receptor (LIR)-1 and cytotoxic T lymphocyte antigen (CTLA)-4 by flow cytometry. Means values ± standard deviation are shown.

TABLE II

Interferon (IFN)-γ and interleukin (IL)-10 production in response to *Trypanosoma cruzi* antigens in patients with chronic Chagas disease

Reference	Test	Source of *T. cruzi* antigen	Incubation time	Findings
Abel et al. (2001)	Capture ELISA with PBMC culture supernatants	Recombinant B13 protein	48 h	IFN-γ levels in CCHD > asymptomatic IL-10 undetectable in all clinical groups
Guedes et al. (2012)	Capture ELISA with PBMC culture supernatants	Trypomastigote lysate (Y strain)	48 h	IFN-γ levels in CCHD > asymptomatic IL-10 levels in asymptomatic > CCHD
Gomes et al. (2003)	FACS intracellular cytokine	Epimastigote/trypomastigote lysate (Y strain)	24 h for IL-10 6 days for IFN-γ	$CD3^+IFN\text{-}\gamma^+$ in CCHD > asymptomatic $CD14^+IL\text{-}10^+$ in asymptomatic > CCHD
Fonseca et al. (2005)	ELISPOT	HLA-A02.01-restricted cruzipain/ FL-160 peptides	24 h	$IFN\text{-}\gamma^+$ cells in CCHD
Gomes et al. (2014)	CBA with PBMC culture supernatants	Epimastigote/trypomastigote lysate (CL strain)	22 h	IFN-γ levels in CCHD = asymptomatic IL-10 levels in asymptomatic > CCHD
Cuellar et al. (2009)	FACS intracellular cytokine	Recombinant KMP-11 *T. cruzi* protein	12 h	$CD4^+IFN\text{-}\gamma^+$ in CCHD > asymptomatic
de Araújo et al. (2012)	FACS intracellular cytokine	Trypomastigote lysate (CL strain)	22 h	Treg $IFN\text{-}\gamma^+$ in CCHD > asymptomatic Treg $IL\text{-}10^+$ in asymptomatic = CCHD
de Melo et al. (2012)	Real time polymerase chain reaction	Recombinant CRA/FRA proteins	3 days	IFN-γ expression in asymptomatic = CCHD IL-10 expression in asymptomatic = CCHD
Albareda et al. (2006)	FACS intracellular cytokine	*T. cruzi*-infected dendritic cells (Brazil strain)	6 h	$CD8^+IFN\text{-}\gamma^+$ in asymptomatic > CCHD
Diez et al. (2006)	ELISPOT	HLA-A02.01-restricted K1 peptide	24 h	$IFN\text{-}\gamma^+$ lymphocytes in asymptomatic = CCHD
Alvarez et al. (2008)	ELISPOT	HLA-A02.01-restricted transialidase peptides	24 h	$IFN\text{-}\gamma^+$ lymphocytes in asymptomatic > CCHD
Fiuza et al. (2009)	FACS intracellular cytokine	Epimastigote lysate (CL strain)	22 h	$CD4CD45RO^{high}IFN\text{-}\gamma^+$ in asymptomatic = CCHD $CD4CD45RO^{high}IL\text{-}10^+$ in asymptomatic = CCHD $CD8CD45RA^{high}IFN\text{-}\gamma^+$ in asymptomatic = CCHD
Lorena et al. (2010)	FACS intracellular cytokine	Recombinant CRA/FRA proteins	24 h	CRA/FRA: $CD4^+IFN\text{-}\gamma^+$ in asymptomatic = CCHD $CD4^+IL\text{-}10^+$ in asymptomatic = CCHD CRA: $CD8^+IFN\text{-}\gamma^+$ in CCHD > asymptomatic $CD8^+IL\text{-}10^+$ in asymptomatic = CCHD
Lasso et al. (2010)	FACS tetramer/intracellular cytokine	HLA-A02.01-restricted K1 peptide	12 h	$CD8^+IFN\text{-}\gamma^+$ cells in asymptomatic = CCHD
Laucella et al. (2004)	ELISPOT	Amastigote enriched lysate (Brazil strain)	24 h	$IFN\text{-}\gamma^+$ cells in asymptomatic > CCHD

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Reference	Test	Source of *T. cruzi* antigen	Incubation time	Findings
Albareda et al. (2009)	FACS intracellular cytokine	Amastigote enriched lysate (Brazil strain)	24 h	$CD4^+IFN\text{-}\gamma^+$ in asymptomatic > CCHD
Marañón et al. (2011)	Bio-Plex with PBMC culture supernatants	HLA-A02.01-restricted HSP-70 peptides	30 h	IFN-γ levels in asymptomatic = CCHD
Egui et al. (2012)	Bio-Plex with PBMC culture supernatants	HLA-A02.01-restricted PFR2/PFR3 peptides	30 h	IFN-γ levels in asymptomatic > CCHD
Gomes et al. (2012)	FACS intracellular cytokine	Live *T. cruzi* (CL strain)	6 h	$IFN\text{-}\gamma^+$ lymphocytes in asymptomatic > CCHD $IL\text{-}10\text{-}\gamma^+$ lymphocytes in asymptomatic > CCHD
Longhi et al. (2014)	Milli-Plex with PBMC culture supernatants	Recombinant P2β/CPO proteins	1-6 days	IL-10 levels increased in CCHD[a]
Longhi et al. (2014)	Milli-Plex with PBMC culture supernatants	Epimastigote lysate (CL Brener, DTU TcVI)	1-6 days	IFN-γ and IL-10 levels increased in CCHD[a]

a: asymptomatic patients were not assessed; CBA: cytometric bead array; CCHD: chronic Chagas heart disease; CRA: cytoplasmatic repetitive antigen; DTU: discrete type unit; FACS: fluorescence-activated cell sorting; FRA: flagellar repetitive antigen; HLA: human leukocyte antigen; HSP-70: heat shock protein-70; KMP-11: kinetoplastid membrane protein-11; PBMC: peripheral blood mononuclear cells; PFR: paraflagellar rod proteins; Treg: regulatory T-cells.

Notably, dissimilar findings have been reported regarding the association of IFN-γ and IL-10 production in response to *T. cruzi* antigens with disease severity during the chronic phase of infection in adult subjects (Table II). In contrast to our findings, increased IFN-γ production after stimulating peripheral blood mononuclear cells (PBMCs) with *T. cruzi* antigens was observed in patients with severe cardiomyopathy compared to seropositive subjects with no signs of cardiac disease (Abel et al. 2001, Guedes et al. 2012), whereas IL-10 production was either detected in asymptomatic patients (Guedes et al. 2012, Gomes et al. 2014) or undetectable (Abel et al. 2001) (Table II). Similarly, patients with severe cardiomyopathy had higher frequencies of $CD3^+$ or $CD4^+$ T-cells, including $CD4^+Foxp3^+CD25hi$ T-cells with the ability to secrete IFN-γ in response to *T. cruzi* antigens (Gomes et al. 2003, Cuellar et al. 2009, de Araújo et al. 2012), whereas IL-10 -secreting cells were found primarily during the asymptomatic phase (Gomes et al. 2003) or detected in all clinical groups (de Araújo et al. 2012). In a recent study focused on Chagas disease patients with severe cardiomyopathy, increased levels of IFN-γ and IL-10 specific for *T. cruzi* antigens have been reported (Longhi et al. 2014).

In contrast to these findings, comparable frequencies of $CD4^+$ T-cells producing IFN-γ and IL-10 were reported in patients with no signs of cardiac disease and those with severe cardiomyopathy (Fiuza et al. 2009). Stimulating PBMCs with cytoplasmatic repetitive antigen (Lafaille 1989, Kriegger 1992) induced IFN-γ, IL-4 and TNF-α production by $CD4^+$ T-cells in patients with or without cardiac disease (Lorena et al. 2010). Additionally, only subjects in the indeterminate phase of Chagas disease presented higher frequencies of $CD4^+IL\text{-}10^+$ T-cells compared with uninfected controls; however, these levels did not differ from those found in patients with severe cardiomyopathy. In contrast, when flagellar repetitive antigen (Lafaille et al. 1989, Kriegger et al. 1992) was used as the antigen source, $CD4^+IL10^+$ T-cells were detected in both patients in the indeterminate phase of the disease and those presenting cardiomyopathy, while the frequencies of $CD4^+IFN\text{-}\gamma^+$ T-cells remained unaltered compared with uninfected controls (Lorena et al. 2010).

In another study, de Melo et al. (2012) reported that most patients in the indeterminate phase of the infection displayed increased IFN-γ gene expression after stimulation with *T. cruzi* antigens, while the majority of patients with cardiomyopathy had high levels of IL-10.

The use of soluble parasite antigens vs. in vitro infection might also lead to different results. In fact, Gomes et al. (2012) recently demonstrated that the short-term in vitro culture of whole blood samples with live *T. cruzi* parasites led to increased $IFN\text{-}\gamma^+$ and $IL\text{-}10^+$ lymphocytes in subjects with no sign of cardiac disease compared with those with severe cardiomyopathy, in contrast to previous reports from the same group showing increased levels of IFN-γ upon stimulation with an epimastigote/trypomastigote lysate (Gomes et al. 2003).

Little information is available regarding $CD8^+$ T-cell responses in subjects with chronic *T. cruzi* infection primarily because the complexity of the *T. cruzi* genome has made it difficult to identify individual targets of $CD8^+$

T-cell responses in *T. cruzi*-infected subjects. In an attempt to identify and quantify T-cell responses to defined *T. cruzi* epitopes, we tested human leukocyte antigen (HLA)-A2.1-restricted $CD8^+$ T-cell responses (HLA-A2.1 is one of the most common class I alleles in this population) to several trans-sialidase-derived epitopes using a combination of HLA-A2 tetramers, ELISPOT analysis and intracellular cytokine staining assays (Martin et al. 2006, Alvarez et al. 2008) (Table II). The percentage of responders strongly correlated with disease severity, with 60% of individuals in the indeterminate phase, but only 22% of the subjects with cardiomyopathy responding to trans-sialidase peptides (Alvarez et al. 2008). When $CD8^+$ T-cell responses were measured using *T. cruzi*-infected autologous dendritic cells, we observed that subjects with less severe forms of the cardiac disease were more likely to mount *T. cruzi*-specific IFN-γ responses than subjects with severe cardiomyopathy (Albareda et al. 2006) (Table II). HLA-A201-restricted epitopes derived from kinetoplastid membrane protein (KMP)-11 (Diez et al. 2006, Lasso et al. 2010), heat shock protein (HSP)-70 (Marañón et al. 2011), FL-160 (Fonseca et al. 2005) or cruzipain (Fonseca et al. 2005) have also been reported as targets of IFN-γ responses by $CD8^+$ T-cells in chronically *T. cruzi*-infected subjects (Table II). While no differences in the magnitude of T-cell responses specific for KMP-11 and HSP-70-derived peptides according to the clinical stage of the disease were found, IFN-γ secretion in response to HLA-A2.01-restricted paraflagellar rod proteins (PRP)-2 and PRP-3-derived peptides was higher in patients in the indeterminate phase of the infection compared with patients with cardiac disease (Egui et al. 2012) (Table II). Concerning the phenotype of $CD8^+$ T-cells specific for *T. cruzi* antigens, $CD8^+$IFN-γ^+ T-cells predominantly showed an effector memory phenotype were enriched in $CD27^+CD28^+$ cells in response to *T. cruzi* (Albareda et al. 2006).

As a whole, Th1 and IL-10-producing T-cells can be detected in the different clinical groups of the chronic infection, with a degree of variation according to the *T. cruzi* antigen source, the patient's location and immunological assays. The quality of T-cell responses might determine the pattern of the cellular response and the severity of the disease in *T. cruzi* infection.

Modulation of *T. cruzi*-specific T-cell responses following chemotherapy

To gain a clearer understanding of the relationship between parasite persistence and *T. cruzi*-specific T-cell maintenance during chronic Chagas disease, we examined the changes in the frequency of *T. cruzi*-specific T-cells in adult subjects with chronic *T. cruzi* infection following BZ treatment. We showed that the levels of IFN-γ-producing T-cells in response to a *T. cruzi* lysate preparation significantly decreased over 12 months following BZ treatment and that IFN-γ-secreting T-cells fell below the level of detection by 36 months post-treatment (Laucella et al. 2009), along with a significant drop in antibody levels specific for a set of recombinant proteins (Laucella et al. 2009, Viotti et al. 2011) as determined by multiplex serological assays (Cooley et al. 2008), in 15 of 32 (47%) patients. This latter assay appeared to be more sensitive than conventional serological tests in detecting changes in antibody levels following BZ treatment. Surprisingly, a proportion of BZ-treated, but not untreated, subjects exhibited an initial increase in IFN-γ responses before the decrease at 12 months post-treatment (Laucella et al. 2009). We hypothesise that this transient rise in IFN-γ-secreting T-cells might be triggered by a release of parasite antigens due to BZ-induced parasite death and might enhance the parasiticidal effect of the drug. The most straightforward interpretation of these results is that BZ treatment decreases the parasite load, thus diminishing the antigen that is required to maintain the *T. cruzi*-specific effector T-cells.

Other studies have measured T-cells responsive to *T. cruzi* antigens after BZ treatment in subjects with chronic *T. cruzi* infection. Of note, these studies focused on the comparison of subjects treated with BZ with those untreated or uninfected; however, these comparisons were not pre-treatment/post-treatment comparisons. A shift towards a type two-modulated profile with a higher level of IL-10 production was found in the $CD4^+$ T-cell compartment, whereas high IFN-γ expression was observed in $CD8^+$ T-cells of *T. cruzi*-infected children after one year of BZ treatment (Sathler-Avelar et al. 2008). Likewise, the treatment of chronically *T. cruzi*-infected adult subjects with no signs of cardiac disease led to increased levels of TNF-α^+CD8^+ and IFN-γ^+CD8^+ T-cells (Sathler-Avelar et al. 2012) with unchanged frequencies of $CD4^+$IL-17^+ T-cells (Guedes et al. 2012) in response to *T. cruzi* antigens. Subjects with chronic *T. cruzi* infection who were treated with BZ 14-30 years earlier and achieved serological cure showed higher levels of IFN-γ production specific for *T. cruzi* compared with patients who had not been cured (Bahia-Oliveira et al. 2000).

In a mouse model of acute and chronic *T. cruzi* infections, BZ treatment resulted in the emergence of *T. cruzi*-specific central memory T-cells (Bustamante et al. 2008). A reduction in the total numbers of activated and TEMs after the treatment of infected mice has also been shown (Fernández et al. 2009). A positive treatment outcome was demonstrated by the observation that treated animals were protected against re-infection (Olivieri et al. 2002). Altogether, changes in *T. cruzi*-specific T-cell responses can be potential early indicators of treatment response, even in cases in which cure is not achieved. Long-term monitoring of T-cell responses following chemotherapy in relation to the serological evolution by conventional serology, which is currently the only gold standard of treatment efficacy in Chagas disease, will allow the usefulness of immunological parameters as surrogate markers of treatment efficacy to be validated.

Immunocompetence as a factor of treatment efficacy in *T. cruzi* infection

The immunological status of the host might also be a determining factor of treatment success. In this respect, a relationship between treatment success and the immune status pre-therapy has been demonstrated in human immunodeficiency virus positive subjects under highly active antiretroviral therapy (Marchetti et al. 2012).

That the immune response can be a key factor in the efficacy of treatment against *T. cruzi* has been documented in experimental acute *T. cruzi* infections; however, strong support of this correlation in *T. cruzi*-infected humans remains lacking. In a murine model of *T. cruzi* infection, treating IFN-γ, IL-12, protein, 55-TNF receptor and inducible nitric oxide synthase knockout mice with BZ reduced the cure rates to 42%, 35% and 28%, respectively, compared with wild-type animals (Romanha et al. 2002). The absence of $CD4^+$ T-cells dramatically affected the efficacy of BZ treatment, whereas parasitaemia and cure rates were less affected by the absence of $CD8^+$ T-cells and B cells (Ferraz et al. 2009). The administration of blocking antibodies for IL-12 or IFN-γ concomitant with a suboptimal dose of BZ enhanced parasitaemia and accelerated mortality, while treatment efficacy did not change in mice treated with optimal doses of BZ (Michailowsky et al. 1998). Simultaneous treatment with a suboptimal dose of BZ and recombinant IL-12 enhanced the efficacy of drug treatment as measured by parasitaemia levels and mouse survival (Michailowsky et al. 1998).

Likewise, BZ treatment during experimental *T. cruzi* infection in mice enhanced phagocytosis, parasite destruction and cytokine release by macrophages (Murta et al. 1999) and led to a partial reversion of spleen and lymph node enlargement observed in *T. cruzi*-infected untreated mice (Olivieri et al. 2006). These findings support the notion that drug treatment responsiveness results from the combined action of the drug and the activation of appropriate immune responses in the host.

Future directions

A throughout knowledge of how drug treatment works might provide guidance regarding how to most effectively treat and monitor therapy outcomes in chronically *T. cruzi*-infected individuals. Providing tools to not only monitor, but also provide early predictions of treatment success or failure will allow the development of new therapeutic options. The notion that improving host immune responses to *T. cruzi* could enhance treatment efficacy remains unexplored in *T. cruzi*-infected subjects and deserves future research.

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